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Brief Articles

Impact of in Vivo Lymphodepletion on Outcome in Children with Nonmalignant Disorders Receiving Peripheral Blood Stem Cell Transplantation

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A B S T R A C T

Peripheral blood stem cell transplantation (PBSCT) with in vivo lymphodepletion can provide faster neutrophil recovery with limited risk of severe graft-versus-host disease (GVHD) in children with nonmalignant disorders (NMDs). We aimed to provide an historical comparison of these 2 strategies regarding the prevalence of GVHD, viral reactivation, timing of immune reconstitution, and final outcomes. Data on 98 children undergoing PBSCT were collected from 5 European pediatric transplantation centers. Only patients with NMDs receiving treosulfan or myeloablative busulfan conditioning and 9-10/10 HLA-matched transplant were included. The patients were divided into 2 groups according to in vivo lymphodepletion with antithymocyte globulin (ATG) or with alemtuzumab. We compared rates of acute and chronic GVHD; Epstein-Barr virus, cytomegalovirus, and adenovirus reactivation; chimerism; lymphocyte recovery; overall survival (OS) and event-free survival (EFS) between the 2 groups. The rate of severe acute GVHD (grade III-IV) was significantly higher in patients receiving ATG (26% vs 10% in alemtuzumab recipients; $P < .05$), whereas viral reactivations occurred with a similar rate in the 2 groups (alemtuzumab, 56%; ATG, 57%). Alemtuzumab was the major risk factor for delayed T cell immune reconstitution in the first 3 months after transplantation (odds ratio [OR], 6.0; 95% confidence interval [CI], 1.8 to 19; $P < .005$). Extended chronic GVHD, ADV reactivation, slower $CD3^+$ cell recovery, and HLA-mismatch reduced the probability of survival. Infections were the main cause of mortality in our cohort, and delayed T cell recovery was significantly associated with mortality in multivariate analysis (OR, 12; 95% CI, 1.2 to 114; $P < .05$). Ultimately, no differences in OS and EFS survival were seen between the ATG and alemtuzumab groups. ATG and alemtuzumab showed similar impacts on outcomes of children undergoing PBSCT for NMDs. The 2 strategies of in vivo lymphodepletion showed specific drawbacks that were counterbalanced by benefits that ultimately led to a comparable survival rate. A patient-centered lymphodepletion strategy can be advised in children undergoing PBSCT for NMDs, by favoring T cell recovery in the presence of invasive infection or GVHD prevention in high-risk mismatched donor transplantation.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) represents the sole curative strategy for several nonmalignant disorders (NMDs). Very good outcomes have been reported with the combination of in vivo T cell depletion and peripheral blood stem cells (PBSCs), with the former preventing graft-versus-host disease (GVHD) and the latter yielding rapid granulocyte recovery. One of 2 main strategies is usually adopted for in vivo lymphodepletion: antithymocyte globulin (ATG) or alemtuzumab (anti-CD52 monoclonal antibody). Comparisons of efficacy between ATG and alemtuzumab as GVHD prophylaxis in children have yielded variable results; the data suggest a greater protective effect against severe acute GVHD (grade III-IV) with alemtuzumab but raise concerns about significantly slower lymphocyte recovery and reduced survival [1,2].

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With the aim of providing an historical data comparison, we report the results of a retrospective multicenter study on PBSC transplantation (PBSCT) in children with NMDs assessing the impact of the 2 strategies of in vivo lymphodepletion.

METHODS

We retrospectively collected data on consecutive PBSCTs in children with NMDs performed in 5 European reference centers. Transplantations performed between December 2007 and December 2017 using myeloablative busulfan- or treosulfan-containing reduced toxicity conditioning [3,4] were included. All procedures in this study were performed in accordance with the ethical standards of the institutional research committees and with the 1964 Declaration of Helsinki and its later amendments. HLA matching was evaluated by high-resolution molecular typing for HLA-A, -B, -C, -DR, and -DQ alleles. The diagnosis of GVHD was made clinically and confirmed pathologically with skin, mucosal, or liver biopsy whenever possible, and grading was performed according to internationally accepted criteria [5,6]. Immune reconstitution was evaluated through absolute counts of lymphocyte subsets by means of flow cytometry at 1, 3, 6, and 12 months after transplantation. Patients underwent weekly PCR analysis of blood for adenovirus (ADV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV) monitoring. Treatment for blood viral infection was administered according to institutional guidelines. Chimerism was evaluated in mononucleated cells in peripheral blood at 1 and 2 years after transplantation or at the last available follow-up. Median values of continuous variables were compared using the Mann-Whitney *U* test, and the log-rank test was used to compare Kaplan-Meier survival curves. To estimate event-free survival (EFS), events were defined as transplant rejection, death, or disease recurrence. Risk factor analyses were performed using Fisher's exact test for univariate analyses and logistic regression for multivariate analyses (including variables associated in univariate analysis with $P < .2$). The threshold for statistical significance was set at $P < .05$.

RESULTS

Data from 98 consecutive patients were retrieved. Patients were divided into 2 groups: recipients of ATG (Grafalon or Thymoglobulin; n = 35) and recipients of alemtuzumab (n = 63). Seven patients received Grafalon at a cumulative planned dose ranging from 30 to 60 mg/kg from day -3 to day -1, and 28 patients received Thymoglobulin with a cumulative planned dose ranging from 6 to 10 mg/kg from day -4 to day -2. Higher doses were used in patients with robust T cell immunity or receiving a 9/10 HLA-mismatched transplant, according to local policies. Alemtuzumab was administered from day -5 to day -1 at a cumulative dose of 1 mg/kg. No significant differences were noted in age at the time of transplantation, underlying disease, HLA matching, or donor type. A significantly higher proportion of patients in the alemtuzumab group received treosulfan-based conditioning and did not receive methotrexate as part of GVHD prophylaxis (Supplementary Table S1).

With a median follow-up of 29 months (range, 1.3 to 128 months), the 2-year overall survival (OS) and EFS of the whole cohort were 85% (95% confidence interval [CI], ± 7.4%) and 80% (95% CI, ± 8%), respectively. No significant difference in OS (ATG, 86%; alemtuzumab, 85%) or EFS (ATG, 83%; alemtuzumab, 79%) were observed, and primary graft rejection was rare in both groups (ATG, 1 of 35 [3%]; alemtuzumab, 1 of 62 [2%]). In the alemtuzumab group, the most common cause of death was viral infection (4 of 8 [50%], including 2 ADV pneumonia, 1 influenza pneumonia, and 1 JC virus infection), with other causes including pneumonitis of unknown etiology (n = 1), treatment-related toxicity (n = 2, including 1 case before stem cell infusion), and vasculopathy (n = 1). In the ATG group, there were 2 deaths due to sepsis and 1 death each from CMV pneumonia, vasculopathy, and severe aGVHD.

Similar frequencies of post-transplantation cumulative ADV, EBV, and CMV viremia necessitating treatment according to institutional guidelines were observed in the 2 groups (57% in the ATG group versus 56% in the alemtuzumab group; $P = .9$). Similarly, each type of viremia occurred in both groups with no significant difference in prevalence (CMV: alemtuzumab, 27% versus ATG, 26% [$P = .9$]; ADV: alemtuzumab, 23% versus ATG, 20% [$P = .9$]; EBV: alemtuzumab, 24% versus ATG, 23% [$P = .9$]). Severe aGVHD (grade III-IV) occurred more frequently with the use of ATG compared with alemtuzumab (9 of 35 [26%] versus 6 of 62 [10%]; $P < .05$) and was not associated with other transplantation-related variables, including HLA matching, conditioning, and GVHD prophylaxis (data not shown). Among the 94 patients evaluable at 100 days of follow-up, 14 (15%) showed signs of cGVHD, which progressed from aGVHD in 11 patients. No statistically significant difference in the prevalence of extensive cGVHD was seen between the 2 serotherapy strategies (ATG, 12%; alemtuzumab, 5%; $P = .2$).

At the last follow-up, a slightly higher proportion of mixed chimerism (donor cells < 95% in whole blood) was seen in the alemtuzumab group (20 of 63 [32%] versus 6 of 35 [17%]), but this difference was not statistically significant ($P = .1$). Similarly, in a subanalysis of patients with Primary Immunodeficiency (PID), the proportion of mixed chimerism was comparable in the 2 groups (ATG, 4 of 16 [25%] versus alemtuzumab, 14 of 43 [33%]). Overall, among patients with mixed chimerism after transplantation, only 3 in the alemtuzumab group experienced disease recurrence: 1 patient with Wiskott-Aldrich syndrome with a low platelet count and developing Inflammatory Bowel Disease (IBD), 1 patient with congenital neutropenia still requiring G-CSF, and 1 patients with LPS Responsive Beige-Like Anchor Protein (LRBA) deficiency with recurrent infections and refractory diarrhea.

Univariate analysis investigating risk factors for mortality detected that survival was negatively impacted by HLA mismatch, ADV reactivation, extensive cGVHD, and delayed T cell recovery ($CD3^+$ <300/mmc at 3 months after transplantation) (Figure 1). Of note, in the multivariate analysis, delayed T cell recovery was the sole independent variable significantly associated with increased mortality rate (odds ratio, 12; 95% CI, 1.2 to 114; $P < .05$) (Supplementary Table S2). A comparison of absolute lymphocyte and subset counts showed significantly higher total lymphocyte and $CD3^+$cell counts in patients receiving ATG up to 3 months after transplantation (+90 day $CD3^+$ cell count, alemtuzumab, 150/mmc versus ATG, 680/mmc; $P <.0001$), whereas $CD3^+$ counts were comparable in the 2 groups at 6 and 12 months. A more striking difference was noted in the $CD8^+$ cell subset, with a faster and greater expansion in the ATG group regardless of viral reactivation. Conversely, the kinetics of recovery of $CD4^+$, $CD19^+$, and $CD16^+/56^+$ cells were comparable in the 2 groups (Figure 2). Logistic regression showed that only the use of alemtuzumab had a significant negative impact on $CD3^+$ cell recovery at 3 months after transplantation (Supplementary Table S3).

DISCUSSION

To the best of our knowledge, this is the largest study of children receiving PBSCT for NMD comparing 2 strategies of in vivo lymphodepletion. The higher $CD34^+$ cell dose infused with PBSCT can facilitate engraftment in nonmalignant transplant recipients [7]. However, previous reports showing significantly higher rates of GVHD and transplantation mortality (TRM) precluded considering PBSCs equivalent to or preferable over bone marrow stem cells in children [8]. In vivo lymphodepletion plays a pivotal role in avoiding transplant rejection and also provides efficient prevention of

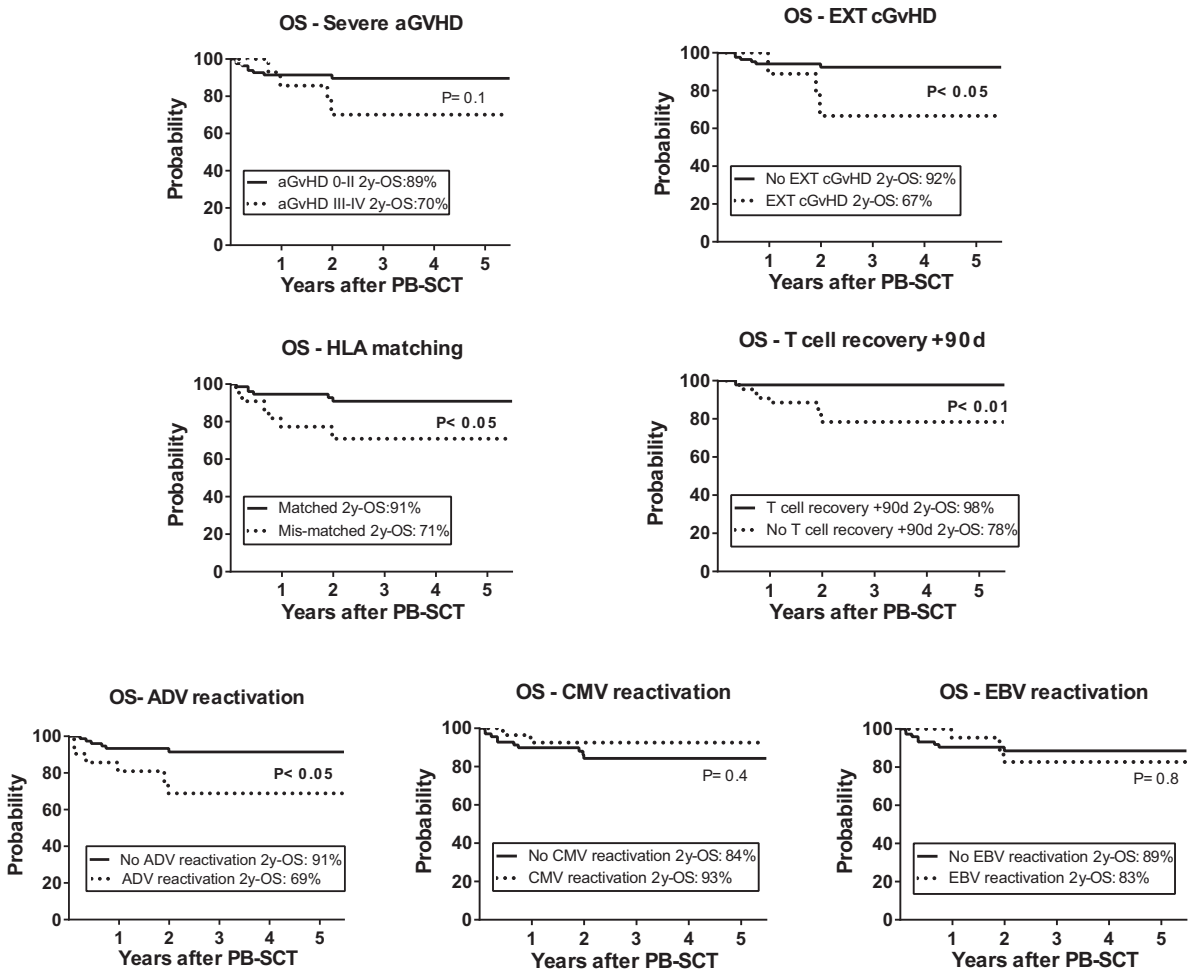

Figure 1. OS of 98 patients undergoing PBSCT with vivo T cell depletion stratified according to occurrence of severe aGVHD (A), occurrence of extensive cGVHD (B), HLA matching (C), T cell recovery at 3 months after transplantation (D), ADV reactivation (E), CMV reactivation (F), and EBV reactivation (G).

GVHD, and the higher doses of $CD34^+$ and $CD3^+$ cells infused with PBSCT promote earlier hematologic recovery. The first important result of this analysis is that both lymphodepleting strategies resulted in a limited TRM and in a very low rate of transplant rejection. This is reassuring, given previous reports documenting a higher mortality rate with alemtuzumab-based conditioning [2].

Overall, our data show that poor $CD3^+$ recovery was associated with a lower survival rate in both groups, and that this was the most significant predictor of mortality. Our data support the advantage of alemtuzumab in effectively mitigating severe aGVHD in children undergoing PBSCT for NMD, for which no benefit from alloimmune reactivity is expected, unlike in malignant diseases. A detrimental effect on early T cell recovery was observed in the alemtuzumab group, which did not translate into worse survival, possibly owing to the limited size of our cohort. Although we are not able to draw definitive conclusions, mainly owing to the retrospective nature of the study and the limited number of patients, especially in the ATG group, it is possible to speculate that the advantages and disadvantages associated with ATG and alemtuzumab could counterbalance negative and positive effects, leading to similar survival rates in the context of PBSCT. Therefore, a patient-tailored decision regarding the optimal in vivo lymphodepletion strategy in this setting might be warranted. In children with active infections at the time of transplantation, the greater risk of mortality associated with delayed immune reconstitution and the negative impact on $CD3^+$ counts of alemtuzumab could support clinicians' decision to use ATG as the lymphodepleting agent. In contrast, in patients with additional risk factors for GVHD (eg, highly mismatched transplants) and a low burden/limited risk of infection, the use of alemtuzumab could confer a protective effect against the occurrence of severe aGVHD and ultimately lead to a better outcome. Nevertheless, it is increasingly clear that drug exposure, more than the type of serotherapy, has the greatest impact on immune reconstitution and GVHD; adjustment of ATG and alemtuzumab exposure, investigated by pharmacokinetics/pharmacodynamics studies, could potentially address patient-tailored dosage targeting with the aim of achieving rapid T cell recovery and reduction of TRM in the setting of PBSCT [2,9,10]. Despite the evident limitations of a retrospective analysis, our data prompt prospective studies to investigate the best approach to lymphodepletion in PBSC transplants in children. Although PBSC is not the currently preferred stem cell source in pediatric patients, broadening the

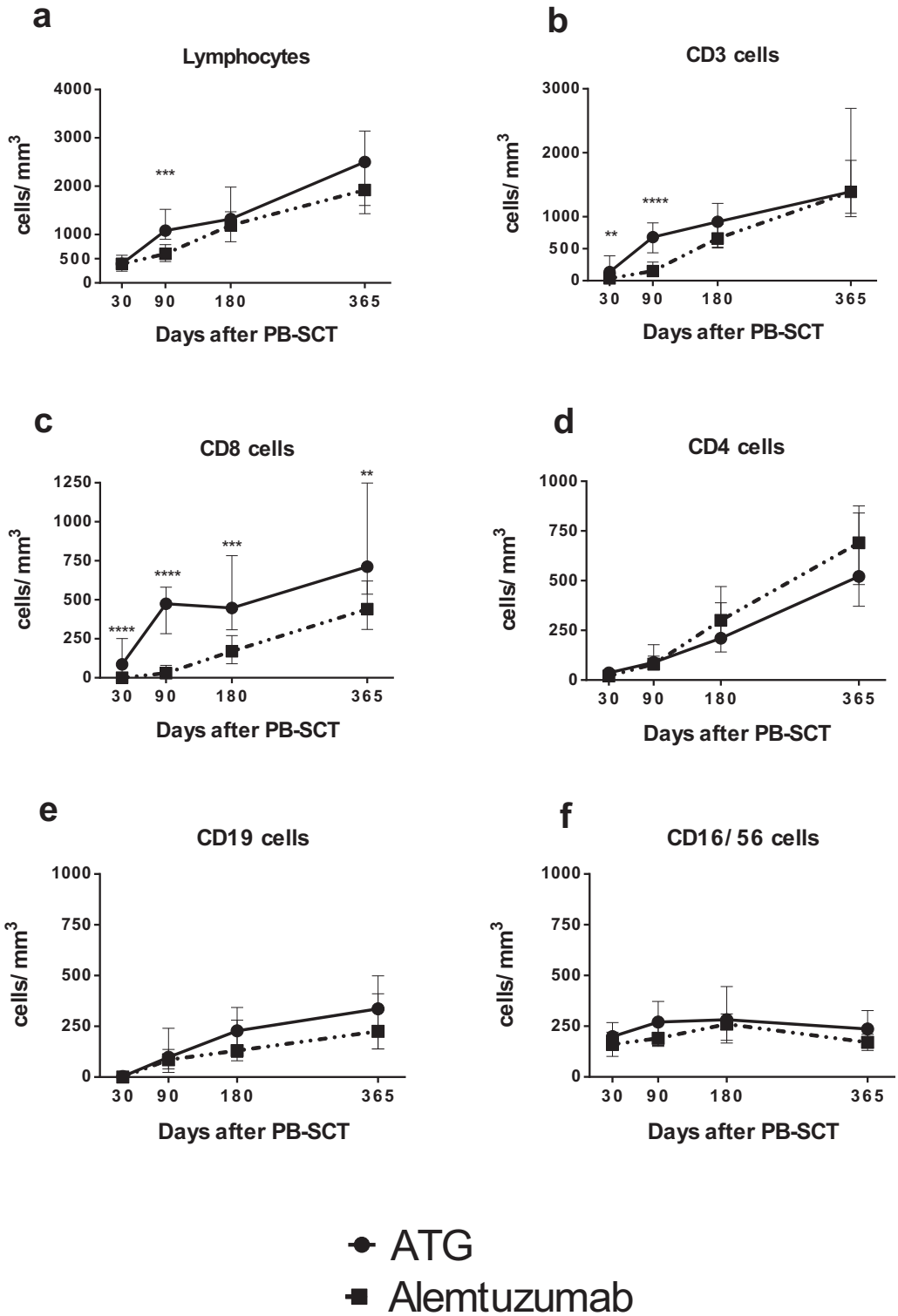

Figure 2. Median values and 95% CIs (whiskers) of lymphocyte count (A) and counts of lymphocyte subsets—$CD3^+$ (B), $CD4^+$ (C), $CD8^+$ (D), $CD19^+$ (E), $CD16/56^+$ (F)—according to the 2 strategies of in vivo lymphodepletion. □, alemtuzumab; •, ATG. $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$.

knowledge in this setting is warranted to guide transplantation planning in those patients who might benefit from a higher $CD34^+$ cell dose or when only PBSCs are available owing to donor choice or barriers to the logistics of bone marrow harvesting.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.08.015.

REFERENCES

1. Shah AJ, Kapoor N, Crooks GM, et al. The effects of Campath 1H upon graft-versus-host disease, infection, relapse, and immune reconstitution in recipients of pediatric unrelated transplants. *Biol Blood Marrow Transplant*. 2007;13:584–593.
2. Willemsen L, Jol-van der Zijde CM, Admiraal R, et al. Impact of serotherapy on immune reconstitution and survival outcomes after stem cell transplantations in children: thymoglobulin versus alemtuzumab. *Biol Blood Marrow Transplant*. 2015;21:473–482.
3. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant*. 2009;15:1628–1633.
4. Slatter MA, Boztug H, Pötschger U, et al. Treosulfan-based conditioning regimens for allogeneic hematopoietic stem cell transplantation in children with non-malignant diseases. *Bone Marrow Transplant*. 2015;50:1536–1541.
5. Martino R, Romero P, Subirá M, et al. Comparison of the classic Glucksberg criteria and the IBMTR Severity Index for grading acute graft-versus-host disease following HLA-identical sibling stem cell transplantation. *Bone Marrow Transplant*. 1999;24:283–287.
6. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2005;11:945–956.
7. Shenoy S, Boelens JJ. Advances in unrelated and alternative donor hematopoietic cell transplantation for nonmalignant disorders. *Curr Opin Pediatr*. 2015;27:9–17.
8. Shimosato Y, Tanoshima R, Tsujimoto S, et al. Allogeneic bone marrow transplantation versus peripheral blood stem cell transplantation for hematologic malignancies in children: a systematic review and meta-analysis. *Biol Blood Marrow Transplant*. 2020;26:88–93.
9. Marsh RA, Lane A, Mehta PA, et al. Alemtuzumab levels impact acute GVHD, mixed chimerism, and lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT. *Blood*. 2016;127:503–512.
10. Sakellari I, Batsis I, Bousiou Z, et al. The role of low-dose anti-thymocyte globulin as standard prophylaxis in mismatched and matched unrelated hematopoietic peripheral stem cell transplantation for hematologic malignancies. *Clin Lymphoma Myeloma Leuk*. 2017;17:658–666.